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CHARACTERIZATION OF SPATIAL REPELLENT, CONTACT IRRITANT, AND TOXICANT CHEMICAL ACTIONS OF STANDARD VECTOR CONTROL COMPOUNDS[1]

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ABSTRACT. A previously described modular high-throughput screening system was used to characterize the spatial repellent, contact irritant, and toxicant chemical actions of 14 compounds historically used or under investigation for vector control. The response of F_1–F_4 *Aedes aegypti* (Thailand strain) to various concentrations of 4 organochlorines (chlordane, DDT, dieldrin, methoxychlor); 4 pyrethroids (alphacypermethrin, cypermethrin, deltamethrin, permethrin); 3 organophosphates (chlorpyrophos methyl, fenitrothion, malathion); 2 carbamates (bendiocarb, propoxur); and 1 pyrazole (chlorfenapyr) were evaluated. Results show chemicals exert different combinations of contact irritant, spatial repellent, and toxic actions. This is true even within the same chemical class. These actions can be ordered for each chemical based on the testing dose at which the specific response is elicited. Data also indicate that behavioral responses to spatial repellent and contact irritant actions are separate (or independent) from the toxic action of a compound. Results from pyrethroid and DDT assays also show chemicals can induce behavior-modifying actions, such as contact irritancy and spatial repellency, which will reduce man-vector contact, despite evidence of insecticide resistance within the test population. These findings support previous laboratory and field studies showing man-vector contact and disease transmission are routinely interrupted by spatial repellent and contact irritant actions of common public health insecticides. Studies similar to that presented here can be used as baseline evidence for expected vector responses and support best approaches for more detailed behavioral research.

KEY WORDS *Aedes aegypti*, vector control, spatial repellency, contact irritancy, toxicity

INTRODUCTION

The ability to quantify chemical actions and the behavioral responses of vectors to those actions is a vital part of discovery of new insecticides, innovative vector control methodologies, and improvements in existing disease control techniques. Historically the search for novel compounds for use in vector control has focused on toxicity or the insecticidal action of chemicals. Studies have shown, however, that there are other chemical actions that break vector-host contact (Muirhead-Thomson 1951; Cullen and DeZulueta 1962; Hamon et al. 1970; Elliott 1972; Gillies 1988; Chareonviriyaphap et al. 1997; Grieco et al. 2000, 2007). Two such actions are contact irritancy and spatial repellency. We define a contact irritant response as the oriented movement of vectors away from a chemical after tarsal contact and spatial repellent response as the oriented movement of vectors away from a chemical without making tarsal contact with chemical residue (Roberts et al. 2000).

Several well-established behavioral assays are currently being used for evaluating chemical actions and for screening of novel compounds. These assays include designs for identifying attraction/attraction inhibition (Kline et al. 2003, Bernier et al. 2005), contact irritancy (WHO 1970, Rutledge et al. 1999, Chareonviriyaphap et al. 2004), noncontact irritancy or excito-repellency (Roberts et al. 1997, Chareonviriyaphap et al. 2002), and anti-biting (Klun and Dubboun 2000) responses of mosquito vectors under laboratory conditions. However, a novel behavioral assay device has recently been developed that can evaluate 3 chemical actions—contact irritancy, spatial repellency, and toxicity—using the same modular system (Grieco et al. 2005). This high-throughput screening system (HITSS) was designed for assaying large libraries of chemicals with the objective of identifying compounds that modify vector behavior, specifically those that could be implemented in insecticide residual spray (IRS) and insecticide-treated net intervention strategies. Recently the HITSS was validated with field data using select compounds in a series of experimental hut trials in Thailand (Grieco et al. 2007).

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Although traditional *Aedes aegypti* L. control has focused on larvaciding, results presented here can be used to improve interventions targeting the adult dengue vector. Recently an international panel met to discuss *Ae. aegypti* control and identify opportunities for increased success (Morrison et al. 2008). The panel emphasized a new control paradigm that shifts primary focus to the adult population using interventions that could be applied within domiciles by the homeowners. This includes novel delivery systems and further development of insecticide-treated materials (Kroeger et al. 2006, Morrison et al. 2008). Such strategies would benefit by integrating knowledge of repellent and/or irritant actions of available vector control compounds into appealing end-user products.

As part of a larger screening program, *Ae. aegypti* was used as the model system in the current study to generate baseline data for comparison with novel compounds because of the ease of rearing large test populations as well as its role as a dengue vector. The following study aimed to 1) use the HITSS assay to characterize contact irritant, spatial repellent, and toxic actions of 14 chemicals historically or proposed (e.g., chlorfenapyr) for use in public health programs; 2) determine the association between testing dose and strength of chemical actions and vector responses; and 3) determine if members of different classes of chemicals exert a similar range of behavioral and toxic actions.

METHODS AND MATERIALS

Detailed descriptions of the HITSS design, mosquito production, assay protocols, and data analysis procedures have previously been published (Grieco et al. 2005, 2007; McLean-Cooper et al. 2008). A complete training manual with assay and insectary protocols can be found at www.usuhs.mil/pmb/TPH/index.html under "Other links of interest," "Behavior Modifying Compounds for Disease Vector Control Training Manual."

HITSS assay device

The HITSS is made up of 5 core modular components that can quickly be reconfigured for each of the 3 assay types: contact irritancy assay (CIA), spatial repellency assay (SRA), and toxicity (TOX) (Fig. 1). These core components include end caps with viewing windows and mosquito introduction portals, a clear cylinder, linking sections with butterfly valves that rotate between an open and closed position, an outer metal cylinder, and an inner metal spool that contains a netting strip treated with a chemical of interest. A complete test unit for CIA is made up of a metal cylinder containing an inner spool with a netting strip linked to a clear cylinder. For SRA, a clear cylinder with 2 metal cylinders attached at either end is referred to as a test unit.

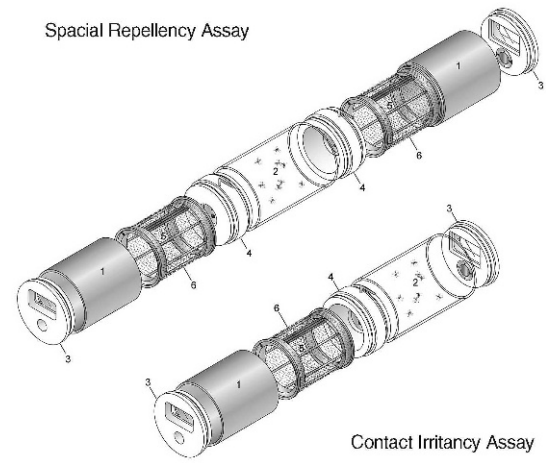

Fig. 1. Schematic drawing of the high-throughput screening system (HITSS) showing the configuration for both spatial repellency (top) and contact irritancy (bottom) assays. The metal treatment cylinder can be used alone to evaluate chemical toxicity. Major components include: 1) treatment cylinder; 2) clear cylinder; 3) end cap; 4) linking sections; 5) inner metal spool; and 6) treatment net (from Grieco et al. 2007).

Mosquitoes

Behavioral assays were performed with *Ae. aegypti* (KAN) colonized at Kasetsart University, Bangkok, Thailand, from larvae collected in Pateu Village, Kachanaburi Province, Thailand (14°20′11″N, 98°59′45″E). The KAN population has been characterized as DDT resistant and pyrethroid tolerant against WHO diagnostic doses (i.e., 4% DDT and 0.25% permethrin) using a standard bottle assay (I. Dusfour personal communication). F_1–F_2 eggs from this population were shipped to the Uniformed Services University of the Health Sciences (USUHS), Bethesda, MD, to establish a colony for mosquito production to be used in the assays. The USUHS colony was maintained until the F_4 generation, at which time a new shipment of F_1–F_2 eggs from Thailand was used to generate a fresh colony population. Mosquitoes for assays were maintained at 27°C, 55% RH, and a light-dark cycle of 12L:12D in an insectary at the Walter Reed Army Institute of Research, Forest Glen, MD. Cohorts of 4- to 7-day-old female *Ae. aegypti* were sorted into individual paper cartons and provided a 10% sucrose solution until 24 h before testing. The numbers of mosquitoes used in the contact irritancy assay (10 females) varied from the number used in both the spatial repellency and toxicity assays (20 females) based on baseline

experiments (data not shown) that were conducted to determine the sample size required for statistical power in the smallest number of replicates with the least difficulty in manual observation. The larger sample size used in the spatial repellency assay was a requirement because of overall lower response levels under nonchemical contact conditions. The sample size for toxicity assays followed guidelines established for insecticide resistance testing (WHO 1998).

Test compounds and exposure concentrations

Assay chemicals were chosen based on historical and/or current use in vector control programs. Chlorfenapyr, developed for agricultural pest control, was also included in evaluations based on current investigations to explore its effectiveness in public health (Mosha et al. 2008). Chemical compounds were acquired as technical grade neat material purchased from Sigma-Aldrich (St. Louis, MO): chlorpyrophos methyl (Catalog no. PS418), cypermethrin (CAS 52315-07-8); DDT (CAS 50-29-3), deltamethrin (CAS 52918-63-5), dieldrin (CAS 60-57-1), malathion (CAS 121-75-5), methoxychlor (CAS 72-43-5), permethrin (CAS 52645-53-1), and propoxur (CAS 114-26-1); or Chem Service Inc. (West Chester, PA): bendiocarb (CAS 22781-23-3), chlordane (CAS 57-74-9), and fenitrothion (CAS 122-14-5). Both alphacypermethrin (CAS 67375-30-8) and chlorfenapyr (CAS 122453-73-0) were provided by BASF (Florham Park, NJ). Netting strips (275 cm^2) were treated with 1.5 ml of a 0.25, 2.5, 25, 250, or 500 $nmol/cm^2$ solution using a micropipette on the morning of each test day. Assay concentrations were based on previous behavioral tests of repellent compounds (Grieco et al. 2005). Netting strips used for control assays were treated with acetone, the chemical solvent, using the same 1.5 ml volume. All nets were allowed to dry for 15 min before placement into individual HITSS inner cylinders. Each chemical net was used for all 3 assays (CIA, SRA and TOX) for a particular chemical and dose conducted during 1 testing day.

Assay protocols

All assays were conducted in a fume hood between 0800 and 1500 h. Laboratory temperatures averaged 24°C (range 23–26°C) with an average 47% RH (range 25–60%). Chambers were washed at the end of each testing day. Sections that contacted the treatment nets (inner spool) were washed using acetone while all other parts were washed using detergent solution (Liqui-Nox, Aloconox, New York, NY). Component sections were allowed to dry overnight before reuse.

Contact irritancy assay

A clear cylinder was connected to a metal test cylinder using a linking section to build 1 test unit. Groups of 10 mosquitoes were introduced into individual test units and allowed to rest for 30 sec. The butterfly valve in the linking section was then placed in the open position for 10 min. The gate was then closed, and the following data collected for each unit: number of specimens within the clear cylinder (i.e., escaping); number of specimens within the metal test cylinder, and knockdown in both clear and metal cylinders. Mosquitoes were considered knocked down if they were observed to be lying on their sides and were unable to right themselves when the chamber was gently tapped. A total of 6 replicates were performed for each chemical and concentration. A matched control was run simultaneously for each replicate.

Spatial repellency assay

A central clear cylinder was connected to 2 metal cylinders (control and treatment) using linking sections to build one test unit. Groups of 20 female mosquitoes were introduced into the clear cylinder and allowed to rest for 30 sec, after which time the butterfly valves were placed in the open position for 10 min. The gates were then closed, and the number of mosquitoes inside each metal cylinder was recorded. Knockdown (KD) response of individuals in the clear cylinder and both metal chambers were also recorded. Nine replicates of each chemical and concentration were conducted.

Toxicity assay

Groups of 20 mosquitoes were held in individual metal test cylinders (i.e., control and treatment) for a 1-h exposure period to evaluate KD action of each chemical. A mosquito was considered knocked down when found lying on its side or back and unable to right itself upon tapping the test chamber. After recording 1 h KD, each group of mosquitoes was then transferred into individually labeled cartons, provided a 10% sucrose solution, and maintained at 27°C and 80% RH to monitor 24-h mortality (MORT) rates. Toxicity assays were performed in 6 replicates for each chemical and concentration. A matched control was run simultaneously for each replicate.

Data analysis

SAS, Base SAS software (1999) v. 8.0 was used for all data analyses. Proportional data were subjected to arcsine square root transformation before statistical analysis. Output tables represent back-transformed values.

CIA: Variations in the percentage of mosquitoes escaping per trial in the CIA were analyzed for each chemical class by 2-way analysis of variance (ANOVA) with 2 main factors (chemical and treatment concentration) and the interaction term for all chemical classes except pyrazole, in which only 1 chemical was tested. Mean percentage escaping was the dependent variable corrected for the number escaping in the control and KD in the metal test chambers. For those chemical classes that showed significant variations, an additional one-way ANOVA was conducted to examine the main factors (chemical or treatment concentration), independently, on percentage escaping. Multiple comparisons of means were done using Scheffe's test ($\alpha = 0.05$). For each chemical dose trial, the difference between the number escaping from treated and control test chambers was analyzed using the Wilcoxon 2-sample test after correction using Abott's formula (1925) as previously described (Grieco et al. 2005, 2007).

SRA: A nonparametric signed-rank test was used for SRA data to determine if a spatial activity index (SAI) was significantly different from zero. The SAI varies from −1 to 1, with zero indicating no response, and is based on the oviposition index of Kramer and Mulla (1979). It is the measure of the proportion of females in the control chamber over the treated chamber after correcting for the proportion of females in the control chamber (Grieco et al. 2005).

TOX: For the toxicity data, the 1 h KD and 24 h MORT rates were corrected based on measurements in control chambers using Abbott's formula (Abbott 1925). Means +/− SE of untransformed data are reported.

RESULTS

Contact irritancy

Within the chemical classes of organochlorine, pyrethroid, and carbamate, there were significant differences in the mean percentage of mosquitoes escaping by chemical and treatment concentration ($P < 0.0001$) (Table 1). Conversely, within the organophosphate chemical class, the mean number of mosquitoes escaping did not significantly vary by chemical or treatment concentration ($P = 0.5838$). Within chemical classes of organochlorines and pyrethroids, the interaction of chemical by treatment concentration was significant ($P < 0.01$), indicating that neither chemical nor treatment concentration can be relied on to be predictive of mean percentage of mosquitoes escaping.

Table 2 shows the responses (corrected percent escape and KD) of mosquitoes in the contact irritancy assay and the association between escape response, chemical, and dose. The mean percentage escaping with corrected percent escape response for all chemicals are available in Tables 3–5. Within the organochlorines, percentage escaping ranged from −10 to 56 (Tables 2–3). There was a highly significant ($P < 0.0001$) association between escape response and dose of DDT, with the highest response observed at a dose of 250 nmoles/cm^2 (Table 2). Escape response was associated with dose of methoxychlor; however, even at the highest dose the response was less than half of that of DDT: 24 versus 56%, respectively. Escape response was significantly associated with dose of dieldrin ($P < 0.05$); however, the range of responses was slightly negative or close to zero and less than the responses observed with DDT at the doses of 25 and 250 nmoles/cm^2 (Table 2). No KD was observed with the organochlorines.

Among pyrethroids, mean percentage escaping ranged from 13 to 93 (Tables 2 and 4). There was a highly significant association between escape response and dose of cypermethrin and deltamethrin ($P < 0.0001$). Interestingly, the highest escape response to cypermethrin was observed at a dose of 250 nmoles/cm^2, whereas the highest response to deltamethrin was observed at 25 nmoles/cm^2 and closely followed by response observed at 2.5 nmoles/cm^2. Treatment concentration did not significantly affect the escape responses with alphacypermethrin ($P = 0.1483$)

Table 1. Results of 2-way ANOVA of the affect of chemical (CHEM), treatment concentration (TRTCON), and the interaction term of these main effects (CHEM*TRTCON) on the mean percentage escaping in the contact irritancy assay by chemical class.

Class/source	df	MS	*F*	*P*
Organochlorine/				
Model	15	1,669.07	11.32	<0.0001
Error	80	147.39		
CHEM	3	3,100.21	21.03	<0.0001
TRTCON	3	2,915.79	19.78	<0.0001
CHEM*TRTCON	9	776.44	5.27	<0.0001
Pyrethroid/				
Model	15	3,599.63	5.67	<0.0001
Error	80	634.83		
CHEM	3	4,374.61	6.89	0.0003
TRTCON	3	8,622.32	13.58	<0.0001
CHEM*TRTCON	9	776.44	5.27	<0.0103
Organophosphate/				
Model	11	165.89	0.86	0.5838
Error	60	193.12		
CHEM	2	227.98	1.18	0.3142
TRTCON	3	276.74	1.43	0.2421
CHEM*TRTCON	6	89.76	0.46	0.8316
Carbamate/				
Model	7	3,556.02	6.00	<0.0001
Error	40	592.39		
CHEM	1	6,643.98	11.22	0.0018
TRTCON	3	4,981.63	8.41	0.0002
CHEM*TRTCON	3	1,101.09	1.86	0.1521

Table 2. Responses of female *Aedes aegypti*[1] in the contact irritancy assay to different chemical and treatment concentrations.

Class and chemical	Mean[2] percentage escaping ± SE by treatment concentration[3] (Mean percent knockdown ± SE)				
	0.25	2.5	25	250	*P*[4]
Organochlorine					
DDT	−6 ± 4Aab[5] (0)	19 ± 8ABa (0)	33 ± 3BCb (0)	56 ± 6Cb (0)	<0.0001
Methoxychlor	8 ± 4ABb (0)	5 ± 4Aa (0)	16 ± 5ABab (0)	24 ± 4Ba (0)	0.0274
Dieldrin	−10 ± 4Aa (0)	3 ± 2ABa (0)	7 ± 3Ba (0)	−2 ± 4ABa (0)	0.0151
Chlordane	−2 ± 2Aab (0)	3 ± 3Aa (0)	2 ± 4Aa (0)	16 ± l0Aa (0)	0.1502
P	0.0113	0.1199	0.0002	<0.0001	
Pyrethroid					
a-cypermethrin	55 ± 18Aa (0)	51 ± 10Aa (2 ± 2)	53 ± 10Aab (15 ± 6)	71 ± 10Ab (37 t 8)	0.1483
Cypermethrin	34 ± 8Aa (0)	30 ± 12Aa (0)	74 ± 7Bab (7 ± 2)	93 ± 7Bab (8 ± 4)	<0.0001
Deltamethrin	31 ± 9Aa (0)	70 ± 4ABa (13 ± 4)	88 ± 5Cb (42 ± 7)	57 ± 3Ba (18 ± 6)	<0.0001
Permethrin	13 ± 15Aa (0)	32 ± 16Aa (0)	56 ± 9Ab (0)	57 ± 10Aa (0)	0.1141
P	0.2057	0.0117	0.0224	0.0019	
Organophosphate					
Fenitrothion	0 ± 4 (0)	1 ± 3 (0)	8 ± 4 (0)	17 ± 3 (0)	NT
Malathion	9 ± 9 (0)	12 ± 3 (0)	9 ± 4 (0)	17 ± 6 (0)	NT
Chlorpyrophos-methyl	10 ± 8 (2 ± 2)	13 ± 7 (2 ± 2)	14 ± 3 (0)	17 ± 7 (0)	NT
P	NT	NT	NT	NT	
Carbamate					
Bendiocarb	10 ± 10Aa (7 ± 7)	49 ± 15ABa (47 ± 12)	67 ± 8Ca (63 ± 6)	41 ± 10ABa (36 ± 8)	0.0131
Propoxur	37 ± 10Aa (5 ± 4)	81 ± 10Ba (64 ± 6)	63 ± 8ABa (50 ± 7)	80 ± 5Bb (53 ± 6)	0.0042
Pyrazole/					
Chlorfenapyr	−10 ± 6 (0)	−5 ± 2 (0)	3 ± 4 (0)	1 ± 3 (0)	0.1259

[1] Four- to 7-day-old, non-blood-fed, 24-h sugar starved, Thai strain.
[2] $n = 6$. For each trial, percentage escaping after correction based on escape in the control and knockdown in the metal test cylinder.
[3] nmoles/cm^2.
[4] All *P* values are from one-way ANOVA examining the effect of treatment concentration or chemical on percentage escaping. NT = not tested.
[5] Means in the same row followed by the same uppercase letter were not significantly different, whereas means in the same column followed by the same lowercase letter were not significantly different. Multiple comparisons of means were done using Scheffe's test ($\alpha = 0.05$).

Table 3. Responses of female *Aedes aegypti*[1] in the contact irritancy assay to different concentrations of organochlorine compounds.

Chemical	Concentration (nmoles/cm^2)	No. of trials (no. mosquitoes)	No. escaping (mean ± SE)		Corrected percentage escaping[2] (mean ± SE)	P[3]
			Treated	Control		
DDT	0.25	6 (60)	0.0 ± 0.0	0.5 ± 0.3	−6 ± 4	0.4545
	2.5	6 (60)	3.5 ± 0.4	1.8 ± 0.5	19 ± 8	0.0519
	25	6 (60)	4.0 ± 0.2	1.0 ± 0.2	33 ± 3	0.0022
	250	6 (60)	6.2 ± 0.6	1.5 ± 0.5	56 ± 6	0.0022
Methoxychlor	0.25	6 (60)	0.8 ± 0.4	0.0 ± 0.0	8 ± 4	0.1818
	2.5	6 (60)	0.5 ± 0.3	0.3 ± 0.2	5 ± 4	1.0000
	25	6 (60)	1.6 ± 0.6	0.0 ± 0.0	16 ± 5	0.0152
	250	6 (60)	3.0 ± 0.2	0.7 ± 0.2	24 ± 4	0.0022
Dieldrin	0.25	6 (60)	0.2 ± 0.2	0.7 ± 0.2	−10 ± 4	0.1515
	2.5	6 (60)	0.3 ± 0.2	0.0 ± 0.0	3 ± 2	0.4545
	25	6 (60)	0.5 ± 0.3	0.3 ± 0.2	7 ± 3	1.0000
	250	6 (60)	0.5 ± 0.2	0.7 ± 0.2	−2 ± 4	1.0000
Chlordane	0.25	6 (60)	0.0 ± 0.0	0.25 ± 0.2	−2 ± 2	1.0000
	2.5	6 (60)	0.3 ± 0.3	0.0 ± 0.0	3 ± 3	1.0000
	25	6 (60)	0.2 ± 0.2	0.3 ± 0.2	2 ± 4	1.0000
	250	6 (60)	2.2 ± 0.9	0.7 ± 0.4	16 ± 10	0.2100

[1] Four- to 7-day-old, non-blood-fed, 24-h sugar starved, Thai strain.
[2] For each trial, percentage escaping after correction using Abbott's formula.
[3] P values are from Wilcoxon 2-sample test for difference between the number escaping in a chemical treatment chamber and an acetone treatment (control) chamber.

and permethrin ($P = 0.1141$) (Table 2). Knockdown ranged from 0 to 42%. Knockdown was observed with all pyrethroids except permethrin, at doses of 2.5 nmoles/cm^2 and above.

Within the organophosphates, mean percentage escaping ranged from 0 to 17. As stated previously (Table 1), mean percentage escaping did not significantly vary ($P = 0.5838$) because of chemical or treatment concentration; therefore no further analyses to compare associations between dose and escape response were conducted. Overall, the organophosphates exhibited very low levels of contact irritancy with significant escape responses occurring only at the higher

Table 4. Responses of female *Aedes aegypti*[1] in the contact irritancy assay to different concentrations of pyrethroid compounds.

Chemical	Concentration (nmoles/cm^2)	No. of trials (no. mosquitoes)	No. escaping (mean ± SE)		Corrected percentage escaping[2] (mean ± SE)	P[3]
			Treated	Control		
α-cypermethrin	0.25	6 (60)	6.7 ± 1.0	1.5 ± 0.6	55 ± 18	0.0087
	2.5	6 (60)	5.8 ± 1.0	2.1 ± 0.4	51 ± 10	0.0119
	25	6 (60)	5.2 ± 0.6	2.0 ± 0.5	53 ± 10	0.0016
	250	6 (60)	5.0 ± 0.4	2.2 ± 0.4	71 ± 10	0.0001
Cypermethrin	0.25	6 (60)	4.2 ± 0.6	1.5 ± 0.3	34 ± 8	0.0108
	2.5	6 (60)	4.3 ± 1.0	1.1 ± 0.3	30 ± 12	0.0280
	25	6 (60)	7.3 ± 0.6	2.0 ± 0.6	74 ± 7	0.0022
	250	6 (60)	8.1 ± 0.6	0.3 ± 0.2	93 ± 7	0.0022
Deltamethrin	0.25	6 (60)	3.5 ± 0.8	0.5 ± 0.3	31 ± 9	0.0087
	2.5	6 (60)	5.8 ± 0.7	0.5 ± 0.2	70 ± 4	0.0022
	25	6 (60)	4.7 ± 0.9	0.5 ± 0.2	88 ± 5	0.0022
	250	6 (60)	3.8 ± 0.6	0.0 ± 0.0	57 ± 3	0.0022
Permethrin	0.25	6 (60)	2.5 ± 0.4	1.2 ± 0.3	13 ± 15	0.6320
	2.5	6 (60)	3.5 ± 1.1	0.3 ± 0.2	32 ± 16	0.0996
	25	6 (60)	5.3 ± 1.6	0.4 ± 0.4	56 ± 9	0.0022
	250	6 (60)	6.1 ± 0.9	0.8 ± 0.4	57 ± 10	0.0022

[1] Four- to 7-day-old, non-blood-fed, 24-h sugar starved, Thai strain.
[2] For each trial percentage escaping after correction using Abbott's formula.
[3] P values are from Wilcoxon 2-sample test for difference between the number escaping in a chemical treatment chamber and an acetone treatment (control) chamber.

Table 5. Responses of female *Aedes aegypti*[1] in the contact irritancy assay to different concentrations of organophosphate (OP), carbamate (CB), and pyrazole (PZ) compounds.

Chemical	Concentration (nmoles/cm^2)	No. of trials (no. mosquitoes)	No. escaping (mean ± SE)		Corrected percentage escaping[2] (mean ± SE)	P[3]
			Treated	Control		
Fenitrothion (OP)	0.25	6 (60)	0.3 ± 0.3	0.3 ± 0.2	−0.4 ± 4	0.1000
	2.5	6 (60)	0.3 ± 0.2	0.7 ± 0.2	1 ± 3	0.1000
	25	6 (60)	1.2 ± 0.3	0.2 ± 0.2	8 ± 4	0.0541
	250	6 (60)	1.5 ± 0.2	0.0 ± 0.0	17 ± 3	0.0022
Malathion (OP)	0.25	6 (60)	2.3 ± 0.5	1.3 ± 0.5	9 ± 9	0.2186
	2.5	6 (60)	1.8 ± 0.4	0.7 ± 0.2	12 ± 3	0.0758
	25	6 (60)	2.2 ± 0.2	0.7 ± 0.4	9 ± 4	0.0455
	250	6 (60)	1.8 ± 0.4	0.3 ± 0.2	17 ± 6	0.0606
Chlorpyrophos-methyl (OP)	0.25	6 (60)	1.2 ± 0.5	0.3 ± 0.2	10 ± 8	0.3182
	2.5	6 (60)	1.2 ± 0.5	0.0 ± 0.0	13 ± 7	0.0606
	25	6 (60)	1.5 ± 0.2	0.3 ± 0.2	14 ± 3	0.0216
	250	6 (60)	1.7 ± 0.5	0.0 ± 0.0	17 ± 7	0.0152
Bendiocarb (CB)	0.25	6 (60)	0.5 ± 0.3	0.2 ± 0.2	10 ± 10	0.7273
	2.5	6 (60)	0.5 ± 0.3	0.2 ± 0.2	49 ± 15	0.1758
	25	6 (60)	0.5 ± 0.3	0.2 ± 0.2	67 ± 8	0.0022
	250	6 (60)	0.3 ± 0.2	0.0 ± 0.0	41 ± 10	0.0022
Propoxur (CB)	0.25	6 (60)	2.8 ± 0.7	0.0 ± 0.0	37 ± 10	0.0022
	2.5	6 (60)	1.8 ± 0.5	0.3 ± 0.2	81 ± 10	0.0022
	25	6 (60)	1.3 ± 0.3	0.0 ± 0.0	63 ± 8	0.0022
	250	6 (60)	3.0 ± 0.8	1.3 ± 0.2	80 ± 5	0.0022
Chlorfenapyr (PZ)	0.25	6 (60)	0.2 ± 0.2	1.0 ± 0.4	−10 ± 6	0.1515
	2.5	6 (60)	0.2 ± 0.2	0.7 ± 0.2	−5 ± 2	0.2424
	25	6 (60)	0.5 ± 0.3	0.3 ± 0.2	3 ± 4	1.0000
	250	6 (60)	0.5 ± 0.2	0.3 ± 0.2	1 ± 3	1.0000

[1] Four- to 7-day-old, non-blood-fed, 24-h sugar starved, Thai strain.
[2] For each trial percentage escaping after correction using Abbott's formula.
[3] P values are from Wilcoxon 2-sample test for difference between the number escaping in a chemical treatment chamber and an acetone treatment (control) chamber.

doses of fenitrothion and chlorpyrophos methyl ($P < 0.05$) and not at all for malathion ($P > 0.05$) (Tables 2 and 5).

The escape response to the carbamates ranged from 10 to 81% (Tables 2 and 5). Both bendiocarb and propoxur treatment concentrations were significantly associated with escape response ($P < 0.05$); however, the doses at which the highest response was observed varied between the chemicals. Knockdown was observed at all treatment concentrations and exceeded 35% at every dose except the lowest, 0.25 nmoles/cm^2.

Escape response to the only pyrazole tested, chlorfenapyr, was low (<4%) and did not significantly vary with dose ($P = 0.1259$) (Tables 2 and 5). No KD was observed with chlorfenapyr (Table 2).

Spatial repellency

Of the 14 chemicals tested, only DDT was observed to have a significant spatial repellent action ($P < 0.001$) for the 3 treatment concentrations $\geq$ 2.5 nmoles/cm^2 (Table 6). Chlorpyrophos methyl also showed significant spatial repellent action, but only at the 25 nmoles/cm^2 concentration ($P < 0.01$). It should be noted that although the spatial repellent action is statistically significant, when weighting the SAI using mean percentage responding, the spatial repellent action of chlorpyrophos methyl (17% responding) is much lower than that of DDT (33% responding). All other compounds were unable to elicit a spatial repellent response even at the highest dose of 250 nmoles/cm^2. Mean percentage responding was highest for DDT (range 7–53%) and the pyrethroids (range 8–32%), with ranges of other chemicals falling between 8 and 24% (Table 6).

Toxicity

The organochlorine compounds and chlorphenapyr resulted in the lowest 1-h KD of all standard compounds assayed (Table 7). Regardless of the treatment concentration, the pyrethroids caused KD of nearly all mosquitoes, whereas KD rates of organophosphates and carbamates varied by dosing levels. Only bendiocarb and propoxur gave consistent modest levels (30–80% range) of KD at treatment concentrations of 0.25 and 2.5 nmoles/cm^2. A treatment concentration of 25 nmoles/cm^2 resulted in nearly 100% mortality for most of the

Table 6. Response of female *Aedes aegypti*[1] in the spatial repellency assay to different chemicals and treatment concentrations.

Class and chemical	Concentration (nmoles/cm^2)	No. of trials (no. mosquitoes)	Mean percentage responding (SE)	Mean SAI[2] (SE)	SR[3]	$P > S$
Organochlorine						
DDT	0.25	9 (180)	7 (2)	−0.05 (0.21)	−1.0	1.0000
	2.5	9 (180)	29 (5)	0.62 (0.12)	38.0	0.0010
	25	9 (180)	33 (1)	0.62 (0.07)	39.0	0.0005
	250	9 (180)	53 (6)	0.49 (0.05)	22.5	0.0039
Methoxychlor	0.25	9 (180)	21 (4)	−0.10 (0.19)	−5.5	0.4766
	2.5	9 (180)	16 (5)	0.37 (0.22)	8.5	0.1719
	25	9 (180)	18 (4)	0.25 (0.22)	6.5	0.3438
	250	9 (180)	17 (2)	0.42 (0.27)	9.5	0.2773
Dieldrin	0.25	9 (180)	12 (5)	0.25 (0.15)	5.5	0.1875
	2.5	9 (180)	7 (2)	−0.29 (0.22)	−7.0	0.4531
	25	9 (180)	17 (3)	−0.24 (0.22)	−7.0	0.2969
	250	9 (180)	11 (3)	0.02 (0.24)	0.5	1.0000
Chlordane	0.25	9 (180)	14 (3)	0.19 (0.26)	6	0.5234
	2.5	9 (180)	9 (2)	−0.22 (0.21)	−3.5	0.4375
	25	9 (180)	12 (3)	0.32 (0.29)	7	0.4531
	250	9 (180)	8 (4)	0.39 (0.16)	5	0.1250
Pyrethroid						
α-cypermethrin	0.25	9 (180)	12 (2)	−0.04 (0.23)	−0.5	1.0000
	2.5	9 (180)	8 (4)	−0.07 (0.12)	0.0	1.0000
	25	9 (180)	15 (3)	0.16 (0.23)	6.5	0.4844
	250	9 (180)	20 (2)	−0.13 (0.21)	−5.5	0.5625
Cypermethrin	0.25	9 (180)	22 (6)	0.22 (0.23)	8.0	0.3711
	2.5	9 (180)	27 (7)	0.12 (0.20)	3.0	0.6719
	25	9 (180)	24 (4)	−0.15 (0.17)	−5.5	0.2813
	250	9 (180)	32 (4)	0.10 (0.20)	5.5	0.5664
Deltamethrin	0.25	9 (180)	19 (3)	−0.03 (0.22)	−1.0	0.9531
	2.5	9 (180)	18 (3)	0.29 (0.19)	10.5	0.1719
	25	9 (180)	25 (3)	0.24 (0.12)	9.5	0.0825
	250	9 (180)	14 (4)	0.29 (0.23)	9.0	0.2422
Permethrin	0.25	9 (180)	25 (5)	0.11 (0.23)	3.5	0.7188
	2.5	9 (180)	21 (4)	−0.02 (0.18)	−2.0	0.7183
	25	9 (180)	32 (8)	0.22 (0.13)	9.5	0.1250
	250	9 (180)	27 (6)	−0.04 (0.18)	−3.0	0.7422
Organophosphate						
Fenitrothion	0.25	9 (180)	16 (4)	0.05 (0.17)	5	0.4688
	2.5	9 (180)	21 (4)	0.07 (0.18)	7	0.1875
	25	9 (180)	14 (2)	−0.05 (0.23)	2.5	0.7188
	250	9 (180)	14 (2)	−0.04 (0.12)	10	0.0938
Malathion	0.25	9 (180)	15 (3)	0.05 (0.17)	0.5	1.0000
	2.5	9 (180)	16 (3)	0.07 (0.18)	2	0.6875
	25	9 (180)	13 (3)	−0.05 (0.23)	−1.5	0.9063
	250	9 (180)	8 (2)	−0.04 (0.12)	0	1.0000
Chlorpyrophos-methyl	0.25	9 (180)	19 (6)	−0.03 (0.06)	−2	0.5000
	2.5	9 (180)	24 (5)	0.02 (0.20)	−1	0.9531
	25	9 (180)	17 (3)	0.74 (0.09)	22.5	0.0039
	250	9 (180)	18 (4)	0.11 (0.30)	18	0.0875
Carbamate						
Bendiocarb	0.25	9 (180)	13 (4)	−0.14 (0.30)	−3	0.7656
	2.5	9 (180)	8 (2)	−0.33 (0.29)	−6	0.4531
	25	9 (180)	21 (4)	0.39 (0.23)	12	0.1797
	250	9 (180)	11 (3)	0.02 (0.14)	0	1.0000
Propoxur	0.25	9 (180)	11 (2)	0.44 (0.25)	11	0.1719
	2.5	9 (180)	11 (2)	0.15 (0.21)	3.5	0.6250
	25	9 (180)	12 (3)	0.0 (0.27)	0	1.0000
	250	9 (180)	24 (4)	0.35 (0.17)	7	0.4531
Pyrazole						
Chlorfenapyr	0.25	9 (180)	13 (3)	0.05 (0.17)	−0.5	1.0000
	2.5	9 (180)	14 (3)	0.07 (0.18)	−4	0.5781
	25	9 (180)	11 (2)	−0.05 (0.23)	−1.5	0.7500
	250	9 (180)	13 (2)	−0.04 (0.12)	−2.5	0.7188

[1] Four- to 7-day-old, non-blood-fed, 24-h sugar starved, Thai strain.
[2] SAI, spatial activity index. See text for details.
[3] SR, signed-rank statistic derived through PROC UNIVARIATE (SAS 1999).

Table 7. Knockdown (KD) and mortality (MORT) of female *Aedes aegypti*[1] to different chemicals and concentrations.

Class and chemical	Concentration (nmoles/cm^2)	No. of trials (no. mosquitoes)	1 h KD[2] (mean % ± SE)	24 h MORT (mean % ± SE)
Organochlorine				
DDT	0.25	3 (60)	2 ± 2	0 ± 0
	2.5	3 (60)	2 ± 2	5 ± 3
	25	3 (60)	0 ± 0	5 ± 5
	250	6 (120)	1 ± 1	15 ± 9
Methoxychlor	0.25	6 (120)	1 ± 1	1 ± 1
	2.5	6 (120)	0 ± 0	8 ± 3
	25	6 (120)	2 ± 2	16 ± 5
	250	6 (120)	24 ± 3	42 ± 3
Dieldrin	0.25	6 (120)	1 ± 1	74 ± 4
	2.5	6 (120)	2 ± 1	89 ± 5
	25	6 (120)	3 ± 3	100 ± 0
	250	NT[3]	NT	NT
Chlordane	0.25	6 (120)	0 ± 0	3 ± 1
	2.5	6 (120)	0 ± 0	0 ± 0
	25	6 (120)	1 ± 1	92 ± 2
	250	6 (120)	17 ± 4	99 ± 1
Pyrethroid				
α-cypermethrin	0.25	6 (120)	73 ± 13	54 ± 6
	2.5	6 (120)	72 ± 18	63 ± 19
	25	6 (120)	98 ± 1	100 ± 0
	250	6 (120)	98 ± 2	100 ± 0
Cypermethrin	0.25	6 (120)	65 ± 13	37 ± 6
	2.5	6 (120)	97 ± 2	92 ± 3
	25	6 (120)	98 ± 1	100 ± 0
	250	6 (120)	97 ± 2	100 ± 0
Deltamethrin	0.25	6 (120)	65 ± 13	37 ± 6
	2.5	6 (120)	97 ± 2	92 ± 3
	25	6 (120)	98 ± 1	100 ± 0
	250	6 (120)	97 ± 2	100 ± 0
Permethrin	0.25	6 (120)	44 ± 13	19 ± 5
	2.5	7 (140)	53 ± 15	27 ± 9
	25	6 (120)	93 ± 4	86 ± 13
	250	6 (120)	97 ± 3	100 ± 0
Organophophate				
Fenitrothion	0.25	6 (120)	7 ± 2	79 ± 7
	2.5	6 (120)	7 ± 2	100 ± 0
	25	6 (120)	66 ± 10	100 ± 0
	250	6 (120)	100 ± 0	100 ± 0
Malathion	0.25	6 (120)	11 ± 3	1 ± 1
	2.5	6 (120)	38 ± 2	97 ± 2
	25	6 (120)	97 ± 3	100 ± 0
	250	NT	NT	NT
Chlorpyrophos-methyl	0.25	6 (120)	9 ± 3	98 ± 2
	2.5	6 (120)	99 ± 4	100 ± 0
	25	6 (120)	100 ± 0	100 ± 0
	250	NT	NT	NT
Carbamate				
Bendiocarb	0.25	6 (120)	78 ± 1	82 ± 7
	2.5	6 (120)	99 ± 1	100 ± 0
	25	6 (120)	99 ± 1	100 ± 0
	250	NT	NT	NT
Propoxur	0.25	6 (120)	36 ± 6	48 ± 5
	2.5	6 (120)	100 ± 1	100 ± 0
	25	6 (120)	99 ± 2	100 ± 0
	250	NT	NT	NT
Pyrazole				
Chlorphenapyr	0.25	6 (120)	1 ± 1	5 ± 1
	2.5	6 (120)	1 ± 1	3 ± 1
	25	6 (120)	0 ± 0	1 ± 1
	250	6 (120)	0 ± 0	3 ± 1

[1] Four- to 7-day-old, non-blood-fed, 24-h sugar starved, Thai strain.
[2] Knockdown and mortality of controls <1% overall.
[3] NT, not tested.

standard compounds tested (Table 7). Exceptions to pattern of high mortalities were DDT, chlorphenapyr, and methoxychlor, for which even highest treatment concentrations (250 nmoles/cm^2) produced mortalities only approximating 15%, 3.0%, and 42.0%, respectively. In contrast, dieldrin, fenitrothion, chlorpyrophos methyl, and bendiocarb exhibited strong toxic actions at the lowest treatment concentration (0.25 nmoles/cm^2), with mortality rates ranging from 74 to 98%.

There were no direct associations between dose of chemical exerting a toxic action and dose exerting either spatial repellent or contact irritant actions (Tables 3–7). For example, DDT had low levels of mortality at the highest test dose (250 nmoles/cm^2), but the same dose of DDT exerted a strong spatial repellent response (Tables 3 and 7). Methoxychlor showed a significant contact irritant escape response at both the 25 and 250 nmoles/cm2 doses, but 1-h exposure to these concentrations resulted in only 16 and 42% mortality rates, respectively. Similar trends were seen with other pyrethroid chemicals.

DISCUSSION

Although chemicals used for vector control have historically been evaluated based on toxicity, characterizing the spatial repellent and contact irritant actions of these compounds is a necessity to further the understanding of the mechanism of action of these important public health tools. Such an understanding will help drive innovative methods for disease control using currently available resources as well as aid in the development of novel compounds.

Data presented in the current study using the HITSS with various concentrations of standard compounds used in vector control programs (as well as chlorfenapyr, currently under investigation for its effectiveness in public health; Mosha et al. 2008) indicate that different chemicals exert different combinations of actions (i.e., spatial repellency, contact irritancy, and toxicity). Results indicate spatial repellency, contact irritancy, and toxicity can vary by dose and between compounds within a single chemical class and that these actions can be expressed independently of one another. For example, results indicate the primary action of DDT is spatial repellency with contact irritancy as the secondary action and toxicity the third. All other compounds evaluated were unable to elicit a spatial repellent response even at the highest dose of 250 nmoles/cm^2. This includes other organochlorines such as methoxychlor, which at one time was a proposed alternative to DDT for IRS (Schoof and Taylor 1972). In contrast, the primary action of alphacypermethrin, and the other pyrethroids tested, is contact irritancy followed by toxicity with no spatial repellency action indicated. Dieldrin's primary action, on the other hand, is toxicity with no contact irritancy or spatial repellency actions indicated. These results have also been validated using the same *Ae. aegypti* Thai strain under field conditions, and similar findings using the HITSS have been reported (Grieco et al. 2005, 2007).

It is understood that the results generated using the *Ae. aegypti* Thai strain do not necessarily translate directly to other vector species, or even different strains of *Ae. aeygpti*, because of behavioral variations that can occur between and among species populations (Potikasikorn et al. 2005, Polsomboon et al. 2008); however, experiments using *Anopheles albimanus* and *Anopheles gambiae* against alphacypermethrin, DDT, and permethrin have indicated similar trends (unpublished data). Further studies using several strains and vector species are warranted to identify the optimal chemical and dose for context-specific interventions. Most importantly, regardless of the vector population tested, these standard compounds have been shown to exhibit repellent and irritant actions despite being characterized as toxicants.

Prioritization of toxic actions over spatial repellent or contact irritant actions brings with it greater risk of rapid selection for resistance to the active ingredient. A balance between one and more of these actions might help reduce selective pressure for resistance to a toxic mode of action. Although the toxicity results with DDT presented here were not unexpected considering predetermined resistance levels of the KAN population using the discriminating dose of 4.0% (~550 nmole/cm2) in the bottle assay (I. Dusfour unpublished data), results of our assay (Grieco et al. 2005) and work of others (Chareonviriphap et al. 1997, Potikasikorn et al. 2005, Polsomboon et al. 2008) show pyrethroid and DDT chemicals can induce contact irritancy and spatial repellency and reduce man-vector contact despite presence of insecticide resistance within test populations.

If contact irritancy and spatial repellency occur independently of toxicity, as our results and others indicate, the likelihood that these behavioral actions would be linked to the resistance status of the vector would be diminished. Therefore, a chemical that altered the house entering and exiting behavior of a vector would do so regardless of resistance level in the target organism. In addition, the current study showed DDT to exert both spatial repellent and contact irritant effects in DDT-resistant test population at doses well below the World Health Organization Pesticide Evaluation Scheme's recommended general field application rate for IRS in malaria control (2 g/m^2 = ~500 nmoles/cm^2; WHO 2001). This rate is based on chemical levels

required for vector mortality. It is well evident that continued evaluations of the relationship between resistance and behavior as well as vector response and chemical dose should be a high priority in vector studies.

Currently pyrethroid and organophosphate chemicals are used for *Ae. aegypti* emergency control through indoor residual or space spray techniques (i.e., thermal fogging and ultra-low-volume spraying) aimed to reduce populations through the toxic action of these compounds (WHO 1997, Gratz 1999). Although such measures have been shown to reduce reported dengue cases during an epidemic for a transient period following application (A. C. Morrison personal communication), they are not routinely integrated into preventive programs. Recent studies, however, have begun to evaluate the effectiveness of pyrethroid-treated curtains in reducing *Ae. aegypti* populations (Kroeger et al. 2006). Results indicate a reduction in densities compared to baseline using the house and Breteau indices, but the effect on the adult densities inside homes (i.e., site of host-vector contact) is unknown.

A household strategy that exploits the repellent and/or irritant actions of currently used insecticides at low doses against *Ae. aegypti*, or other vector species, could be incorporated into a consumer product mechanism (pretreated material strips, tiles, wallpaper, paint, etc.) to prevent house entry or promote early exiting of the vector prior to taking a blood meal. Such an approach may decrease cost because of lower levels of active ingredient required, increase sustainability of an intervention through home ownership, and broaden the delivery platforms of available chemicals for traditional house treatment. The effect of a repellent/irritant based intervention on dengue transmission will need to be evaluated using a combination of entomological (to include adult measures) and serological surveys.

In conclusion, vector control strategies continue to place the primary focus of a chemical's effectiveness on its toxic action to the exclusion of spatial repellency and contact irritancy. The current study continues to support the fact that the impact of public health insecticides on vector populations is much more complex than just toxicity. This emphasis on toxicity precludes development and use of many compounds and control strategies (novel or established) that could reduce vector-host interaction. It should be preferred that the compounds tested in the current research were selected based on their use in public health and/or agricultural programs. These compounds therefore have been labeled according to the paradigm of degree of toxic action; however, data presented here show these insecticides also exert spatial repellent and contact irritant actions. It is the belief of the authors that if screening programs adopted an approach that uses a random search of chemical libraries for biological activity other than toxicity, a much wider range of actions would be identified, and the independence of these actions from toxicity would be more apparent. Such an approach is a vital component in driving innovative research. For this reason, screening programs need to be established that include spatial repellent and contact irritant actions as a focus of lead discovery in insecticide development efforts. Focusing entirely on the toxic action of these compounds has delayed the expansion of our vector control arsenal as well as the options for novel vector control strategies using readily available public health tools.

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